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Carcinogenic Tryptophan Pyrolysis Products Potent Inhibitors of Type A Monoamine Oxidase[1]) and the Platelet Response to 5-Hydroxytryptamine[2])

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Summary: The effects of carcinogenic heterocyclic amines and β-carbolines on 5-hydroxytryptamine-induced human platelet aggregation, on the uptake of 5-hydroxytryptamine by platelets, and on human monoamine oxidase activity were investigated. Of the dietary carcinogens and β-carbolines studied, carcinogenic tryptophan pyrolysis products had greater pharmacological activities than other heterocyclic amines. The carcinogenic tryptophan pyrolysis products, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, which have been identified in the dialysis fluid of uraemic patients, were the most potent inhibitors of the aggregation response to 5-hydroxytryptamine, with IC_{50} (the concentrations causing 50% inhibition) values of 10 μmol/l and 50 μmol/l, respectively. 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole by themselves did not induce platelet aggregation, although these dietary carcinogens structurally resemble 5-hydroxytryptamine. Kinetic analyses showed that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole were potent competitive inhibitors of 5-hydroxytryptamine uptake by platelets with K_i 18 μmol/l and 42 μmol/l, respectively. Furthermore, carcinogenic tryptophan pyrolysates as well as β-carbolines were found to be competitive selective inhibitors of monoamine oxidase 'type A'.

Introduction

During the last decade, a new series of heterocyclic amines has been isolated as potent mutagens from pyrolysates of amino acids and proteins and from broiled fish and meat (1, 2). All the mutagenic heterocyclic amines so far tested have been shown to be carcinogenic in experimental animals (1, 2). Recently, some mutagenic and carcinogenic heterocyclic amines such as 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole and 2-aminodipyrido[1,2-a:3′,2′-d]imidazole were identified in the dialysis fluid of patients with uraemia, and in human plasma (3–6).

Among such carcinogens, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole and γ-carboline derivatives, strongly inhibit human platelet aggregation by inhibiting prostaglandin endoperoxide synthetase (7). In the course of the previous study, we found that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole are also potent inhibitors of 5-hydroxytryptamine-induced human platelet aggregation. Since aggregation induced by 5-hydroxytryptamine is mediated by a specific receptor (8, 9), it is perhaps relevant that the tryptophan pyrolysis products, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, are structurally related to 5-hydroxytryptamine (serotonin).

On the other hand, β-carbolines such as tetrahydro-β-carboline have been reported to be selective inhibitors of monoamine oxidase A (EC 1.4.3.4) (10–12).

[1]) Enzyme: Monoamine oxidase (amine: oxygen oxidoreductase, deaminating, flavin-containing; E.C. 1.4.3.4)

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Furthermore, tetrahydro-β-carboline and/or its derivatives which structurally resemble 5-hydroxytryptamine were shown to be potent uptake inhibitors of 5-hydroxytryptamine in human platelets and mouse brain (11, 13). These findings suggested us that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole may be not only 5-hydroxytryptamine uptake inhibitors but also potent monoamine oxidase inhibitors. In the present report, we describe the effects of carcinogenic tryptophan pyrolysates on 5-hydroxytryptamine-induced platelet aggregation, human platelet 5-hydroxytryptamine uptake and human monoamine oxidase activity in vitro. The chemical structures of the heterocyclic amines including β-carbolines used in the present study are shown in figure 1.

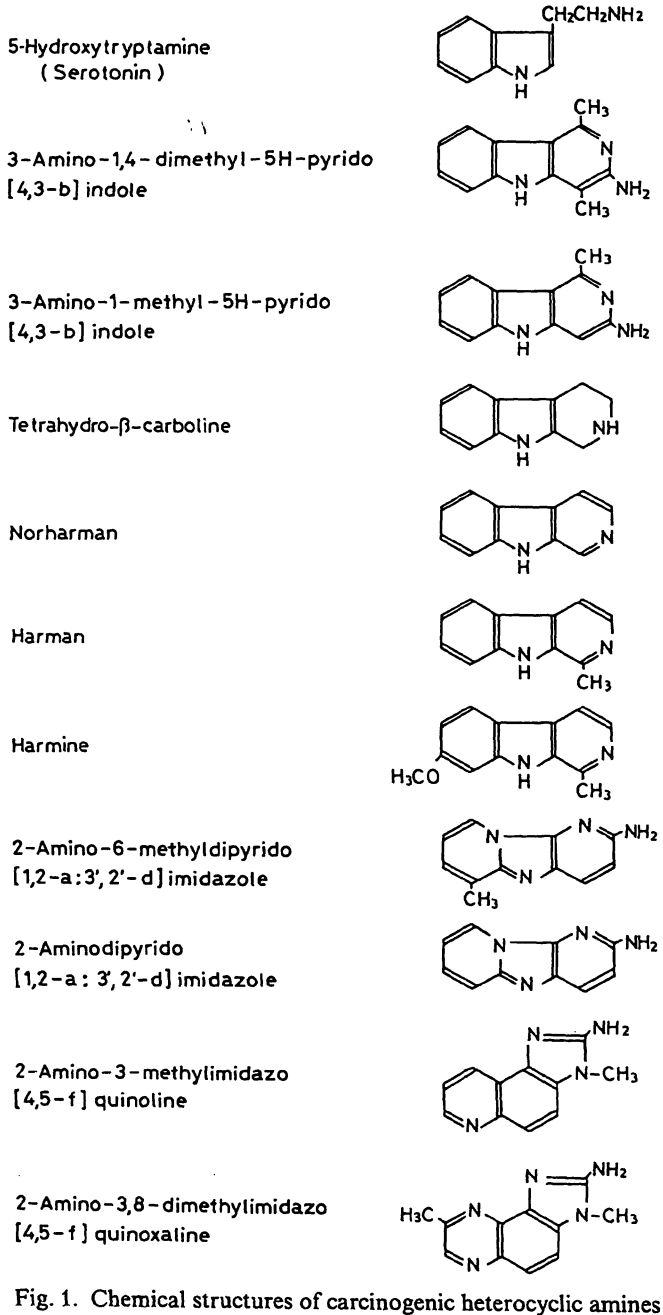

Fig. 1. Chemical structures of carcinogenic heterocyclic amines and β-carbolines used in the present study.

Materials and Methods

Preparation of platelet-rich plasma

Human blood (9 vol) was collected in 1 vol of 38 g/l trisodium citrate as an anticoagulant. The citrated blood was gently mixed and centrifuged at 180 *g* for 20 min at 20 °C, and the supernatant (platelet-rich plasma) was carefully transferred with a plastic pipette into plastic tubes. For the preparation of platelet-poor plasma, the remainder of the blood was centrifuged at 3000 *g* for 15 min. For platelet aggregation studies, the platelet concentration of platelet-rich plasma was adjusted to 2–5 × 10^{11}/l by addition of an appropriate amount of platelet-poor plasma (14).

Platelet aggregation

This was performed at 37 °C using a Chronolog aggregometer (Chronolog Co., Havertown, PA, U.S.A.) (7, 14, 15). Aggregation was measured as percent light transmission, using the light transmission of platelet-rich plasma as 0% and that of platelet-poor plasma as 100%. Platelet aggregation was initiated using 5-hydroxytryptamine and ADP. Added chemicals consisted of 10% of the final volume of the assay mixture (14).

Hererocyclic amines were dissolved in saline and added to platelet-rich plasma just before the addition of the stimulator.

5-Hydroxytryptamine uptake studies

The uptake of [^{14}C]5-hydroxytryptamine into human platelets was determined by the method of *Omen & Smith* (16) with the following modification. Platelet-rich plasma was prepared from whole blood in sodium citrate instead of using EDTA, and platelet-rich plasma was diluted with saline instead of platelet-poor plasma, because the latter contains unknown inhibitors of 5-hydroxytryptamine uptake (17). The average platelet counts in all experiments were in the range of 6–8 × 10^{11}/l and the specific activity of the [^{14}C]5-hydroxytryptamine used was 7200–8400 Bq/pmol. Platelet-rich plasma was incubated at 37 °C in the small polypropylene centrifuge tubes with radioactive 5-hydroxytryptamine with or without the heterocyclic amines at different concentrations. The final volume in each tube was 1 ml. After incubation, the tubes were centrifuged in the Beckman Microfuge at 20 000 *g* for 30 s. The supernatant was decanted and the inside of the tube above the platelet pellet was wiped with cotton-tipped applicators to remove final traces of plasma. The pellet was resuspended in 200 µl water and frozen and thawed twice to lyse the platelets and to release their 5-hydroxytryptamine (8). The lysate was transferred into the vial of a scintillation counter. Each vial received 10 ml Scintisol EX-H (Dojindo Laboratories, Kumamoto, Japan) and radioactivities were estimated by liquid scintillation counting. The radioactivity of the sample which was incubated at 0 °C was defined as the non-specific [^{14}C]5-hydroxytryptamine uptake. The specific uptake was calculated as total minus non-specific uptake. Preliminary experiments showed in linear relationship between the incubation time (1 min to 20 min) and the amount of incorporated radioactive 5-hydroxytryptamine. Therefore, in subsequent experiments, radioactive 5-hydroxytryptamine was incubated with platelet-rich plasma for 5 min.

Sources of monoamine oxidase

Human platelets were used as a source of monoamine oxidase B and human placenta as a source of monoamine oxidase A (18, 19). Platelet-rich plasma (20 ml), prepared as described above, was centrifuged at 3000 *g* for 10 min. The supernatant was removed by decantation and the platelet pellets were washed with 0.32 mol/l sucrose by suspension and resedimentation at 3000 *g*. The pellets were finally resuspended in 2 ml of 0.32 mol/l sucrose and finally sonicated with an ultrasonic disintegrator for 30 s and frozen at −20 °C for future use (20).

Fresh human placenta (760 g) was washed three times with ice cold saline. An homogenate of placenta (100 g, made up with 10 mmol/l phosphate buffer, pH 7.4 to 1 liter) was prepared and frozen at −20 °C until use (12). Before the monoamine oxidase assay, an aliquot was diluted twenty-fold with 100 mmol/l phosphate buffer at pH 7.4.

Monoamine oxidase assay

The reaction mixture contained 410 µl of 50 mmol/l phosphate buffer, pH 7.4, 50 µl of heterocyclic amine solution in 50 mmol/l phosphate buffer, 20 µl of monoamine oxidase preparation and 20 µl of substrate solution. Controls contained 50 µl of phosphate buffer in place of the heterocyclic amine solution. [^{14}C]5-Hydroxytryptamine and [^{14}C]phenylethylamine were diluted with non-radioactive substrate. Mixtures were incubated for 30 min at 37 °C and the reaction stopped on ice followed by the addition of 100 µl of 2 mol/l citric acid (12). The labelled products of the reaction were extracted into 3 ml of toluene/ethylacetate (1 + 1, by vol) when 5-hydroxytryptamine was used as substrate, and into 3 ml of toluene for phenylethylamine (12). Radioactivities were measured by liquid scintillation counting. All assays were carried out in duplicate and concentrations of heterocyclic amines causing 50% inhibition of [^{14}C]5-hydroxytryptamine and [^{14}C]phenylethylamine deamination by monoamine oxidase (IC_{50}) were determined graphically. The concentrations of [^{14}C]5-hydroxytryptamine and [^{14}C]phenylethylamine in the incubation medium were 10.4 µmol/l and 11.4 µmol/l, respectively.

Materials

5-Hydroxy[side-chain 2-^{14}C]tryptamine-creatinine sulphate (2.11 TBq/mol ≙ 57.0 mCi/mmol) and 2-phenyl[1-^{14}C]ethylamine hydrochloride (2.22 TBq/mol ≙ 60.0 mCi/mmol) were from Amersham International (Amersham, U. K.). 5-Hydroxytryptamine creatinine sulphate complex and β-phenylethylamine hydrochloride were from Wako Pure Chemical Industries (Osaka, Japan). Harman hydrochloride, norharman and tetrahydro-β-carboline were from Sigma Chemical Co. (St. Louis, MO, U. S. A.). 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate, 3-amino-1-methyl-5H-pyrido[4,3-b]indole acetate, 2-amino-6-methyldipyrido[1,2-a:3′,2′-d] imidazole hydrochloride and 2-aminodipyrido[1,2-a:3′,2′-d]imidazole hydrochloride were from Wako Pure Chemical Industries. 2-Amino-3-methylimidazo[4,5-f]quinoline hydrochloride and 2-amino-2,8-dimethylimidazo[4,5-f]quinoxaline hydrochloride were kindly provided by Dr. *Shigeaki Sato*, National Cancer Center Research Institute (Tokyo, Japan). All other chemicals were of analytical grade.

Results

Effects of heterocyclic amines on human platelet aggregation

It is well known that 5-hydroxytryptamine induces a transient reversible platelet aggregation in 90% of a normal population (8, 21), while the platelet aggregation response to ADP is biphasic and irreversible at high doses of the stimulant. Aggregation induced by these stimulants is mediated by specific receptors. The K_m value of the platelet aggregation response to 5-hydroxytryptamine was determined from the initial rate of aggregation induced by concentrations of 5-hydroxytryptamine ranging from 1.0 − 20 µmol/l (11).

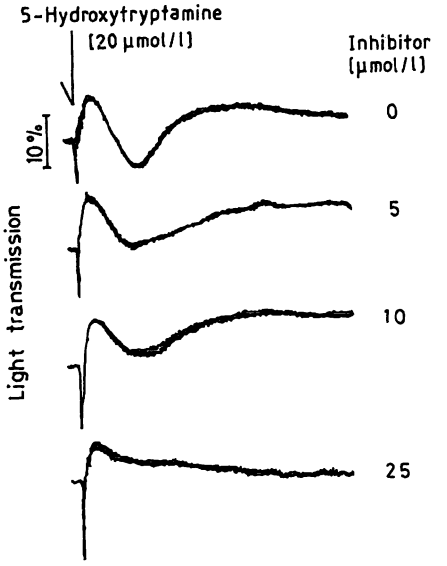

Fig. 2. Inhibitory effects of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole on human platelet aggregation induced by 5-hydroxytryptamine. The extent of aggregation was measured as percent light transmission at 610 nm. Parentheses indicate the final concentrations of the inhibitor (µmol/l).

The determined K_m value was 0.38 µmol/l, which agrees with the value reported previously (11). As shown in figure 2, the inhibitory effects of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole on 5-hydroxy-

Tab. 1. Effects of heterocyclic amines on 5-hydroxytryptamine-induced human platelet aggregation

Compounds	K_m (µmol/l)	IC_{50}* (µmol/l)
5-Hydroxytryptamine	0.38	—
Carcinogenic heterocyclic amines		
3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	—	10
3-Amino-1-methyl-5H-pyrido[4,3-b]indole	—	50
2-Amino-6-methyldipyrido [1,2-a:3′,2′-d]imidazole	—	150
2-Amino-dipyrido [1,2-a:3′,2′-d]imidazole	—	150
2-Amino-3-methyl-imidazo[4,5-f]quinoline	—	No inhibition (100)**
2-Amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline	—	No inhibition (100)**
β-Carbolines		
Tetrahydro-β-carboline	—	150
Norharman	—	200
Harman	—	200
Harmine	—	170

* Concentrations of compounds causing 50% inhibition of 5-hydroxytryptamine (20 µmol/l)-induced platelet aggregation.

** The maximum concentration (µmol/l) used for the experiment.

tryptamine-induced platelet aggregation was dose-dependent. The IC_{50} (the concentration causing 50% inhibition) for platelet aggregation induced by 5-hydroxytryptamine was estimated graphically. Among the heterocyclic amines tested in this study, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole was the most potent inhibitor, with an IC_{50} of 10 μmol/l (tab. 1). 3-Amino-1-methyl-5H-pyrido[4,3-b]indole, tetrahydro-β-carboline and harmine were less potent inhibitors than 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole. 2-Amino-3-methylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline did not show significant effects on 5-hydroxytryptamine-induced platelet aggregation, even at a concentration of 100 μmol/l.

Although these heterocyclic amines structurally resemble 5-hydroxytryptamine (fig. 1), none of them was capable of inducing platelet aggregation, even at a high concentration as 100 μmol/l. In addition, we reconfirmed that these heterocyclic amines did not affect the first wave ADP-induced aggregation, as reported previously (7).

Effects of heterocyclic amines on 5-hydroxytryptamine uptake by human platelets

We also examined the effect of heterocyclic amines on 5-hydroxytryptamine uptake by platelets, not only because the carcinogenic heterocyclic amines structurally resemble 5-hydroxytryptamine (fig. 1), but also because some of the heterocyclic amines examined in this study had potent inhibitory effects on the platelet aggregation response to 5-hydroxytryptamine. In the present system, the K_m for 5-hydroxytryptamine uptake was 1.6−2.2 μmol/l (1.8 ± 0.25 μmol/l, mean ± S.D., n = 4) with a V_{max} of 16.5 pmol/5 min per 10^7 platelets. These results are slightly higher than those reported previously (11, 16). This may be due to differences in the anticoagulant used, the extraction procedure for radioactive 5-hydroxytryptamine, or the dilution medium used for the platelet-rich plasma.

It was confirmed by kinetic analysis that the inhibition by 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole is competitive with 5-hydroxytryptamine, with a K_i value of 18 μmol/l. Other heterocyclic amines such as 3-amino-1-methyl-5H-pyrido[4,3-b]indole, tetrahydro-β-carboline and harmine were less potent inhibitors than 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole. The K_i values are summarized in table 2; 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole is the most potent inhibitor among the heterocyclic amines studied.

Tab. 2. Effects of heterocyclic amines on platelet 5-hydroxytryptamine uptake.

Compounds	K_m (μmol/l)	Uptake K_i (μmol/l)	Type of Inhibition
5-Hydroxytryptamine	1.8	–	
Carcinogenic heterocyclic amines			
3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	–	18	competitive
3-Amino-1-methyl-5H-pyrido[4,3-b]indole	–	42	competitive
2-Amino-6-methyl-dipyrido [1,2-a:3′,2′-d]imidazole	–	–	No inhibition (100)*
2-Aminodipyrido [1,2-a:3′,2′-d]imidazole	–	–	No inhibition (100)*
2-Amino-3-methyl-imidazo[4,5-f]quinoline	–	–	No inhibition (100)*
2-Amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline	–	–	No inhibition (100)*
β-Carbolines			
Tetrahydro-β-carboline	–	55	competitive
Norharman	–	90	competitive
Harman	–	95	competitive
Harmine	–	72	competitive

* The maximum concentration (μmol/l) used for the experiment.

Effects of heterocyclic amines on monoamine oxidase

Monoamine oxidase is now classified into two types, monoamine oxidase-A and -B, according to their sensitivity to the specific inhibitors and their substrate specificities. β-Carbolines such as harmine and harman as well as other structural analogues of the harman series are known to be reversible and competitive inhibitors of monoamine oxidase-A (10−13). Human platelets have been shown to contain almost exclusively the B type, whereas human placenta contains only the A type (18, 19). Therefore, human placenta was used as a source of pure monoamine oxidase-A and human platelets as a source of monoamine oxidase-B. Using the monoamine oxidase-A-selective substrate, 5-hydroxytryptamine, and the monoamine oxidase-B-selective substrate, phenylethylamine, the IC_{50} values were determined for the inhibition of monoamine oxidase-A and -B. Figure 3 shows the inhibition by heterocyclic amines of the monoamine oxidase-A activity of human placenta homogenate towards 10.4 μmol/l 5-hydroxytryptamine. The curves obtained with the heterocyclic amines indicate the 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, tryptophan pyrolysis products, were more potent monoamine oxidase-A inhibitors than other carcinogenic heterocyclic amines including 2-

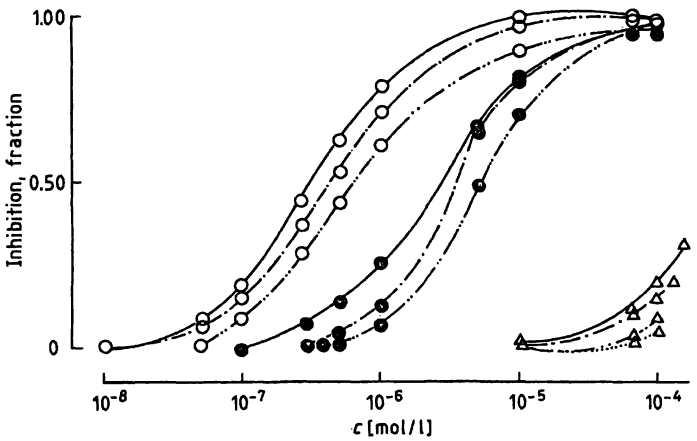

Fig. 3. Inhibition of monoamine oxidase A activities in human placenta homogenate, using 10.4 μmol/l 5-hydroxytryptamine, by carcinogenic heterocyclic amines and β-carbolines.

o—o	= harmine
o—·—o	= 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole
o—··—o	= 3-amino-1-methyl-5H-pyrido[4,3-b]indole
●—●	= harman
●———●	= norharman
●———●	= tetrahydro-β-carboline
△—△	= 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole
△———△	= 2-aminodipyrido[1,2-a:3′,2′-d]imidazole
△———△	= 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
△-----△	= 2-amino-3-methylimidazo[4,5-f]quinoline

Tab. 3. Effects of heterocyclic amines on human placenta and platelet monoamine oxidase (MAO) activities.

Heterocyclic Amines	IC_{50}* (μmol/l)	
Carcinogenic heterocyclic amines	*5-Hydroxytryptamine + placenta MAO*	*Phenylethylamine + platelet MAO*
3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	0.45	> 200
3-Amino-1-methyl-5H-pyrido[4,3-b]indole	0.70	> 200
2-Amino-6-methyldipyrido [1,2-a:3′,2′-d]imidazole	> 150	> 150
2-Aminodipyrido [1,2-a:3′,2′-d]imidazole	> 150	> 150
2-Amino-3-methylimidazo[4,5-f]quinoline	> 100	> 100
2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline	> 100	> 100
β-Carbolines		
Tetrahydro-β-carboline	5.00	> 150
Norharman	3.00	70
Harman	2.50	80
Harmine	0.35	60

* IC_{50} is the concentration of heterocyclic amine required to yield 50% inhibition of 10.4 μmol/l 5-hydroxytryptamine or 11.4 μmol/l [^{14}C]phenylethylamine deamination. Human placenta was used as a source of monoamine oxidase-A and human platelets as a source of monoamine oxidase-B.

amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole, 2-aminodipyrido[1,2-a:3′,2′-d]imidazole, 2-amino-3-methylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. The IC_{50} values for the inhibition of monoamine oxidase-A and -B were summarized in table 3. The results shown in table 3 apparently indicate that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole are selective monoamine oxidase-A inhibitors.

Discussion

The results demonstrate that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, carcinogenic tryptophan pyrolysis products, have potent inhibitory effects on the human platelet aggregation response to 5-hydroxytryptamine, 5-hydroxytryptamine uptake by human platelets and monoamine oxidase-A activities. Among the carcinogenic heterocyclic amines studied in this experiment, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole showed greater pharmacological activities than other carcinogenic heterocyclic amines such as 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole, 2-aminodipyrido[1,2-a:3′,2′-d]imidazole, 2-amino-3-methylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. This may be explained by the fact that tryptophan pyrolysis products, like many inhibitors of 5-hydroxytryptamine metabolism, are related to 5-hydroxytryptamine (fig. 1). In this investigation, however, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole had more potent inhibitory effects on 5-hydroxytryptamine-induced platelet aggregation and platelet 5-hydroxytryptamine uptake than β-carbolines, which structurally resemble 5-hydroxytryptamine (fig. 1, tab. 1 and 2). The reason was not clear, but an amino-group of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole might play an important role in their inhibitory actions on the platelet response to 5-hydroxytryptamine (fig. 1). On the other hand, β-carbolines such as tetrahydro-β-carboline, harman and harmine as well as other structural analogues of harman series are known to be reversible and competitive inhibitors of monoamine oxidase-A (10—13). In this study, we reconfirmed that β-carbolines were selective monoamine oxidase-A inhibitors. Moreover, we found that 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, and γ-carbolines were also potent inhibitors of monoamine oxidase-A (fig. 3 and tab. 3). The results

showed that 3-amino-1,4-dimethyl-5H-pyrido-[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole were more potent inhibitors of monoamine oxidase-A than β-carbolines, with the exception of harmine. However, we could not provide a satisfactory explanation for the observation that harmine was the most potent inhibitor of monoamine oxidase-A in the heterocyclic amines studied (tab. 3). From the viewpoint of the chemical structures, further studies are needed.

Recently, various carcinogenic heterocyclic amines including 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole and 2-aminodipyrido[1,2-a:3′,2′-d]imidazole were identified in the dialysis fluid of uraemic patients and in human plasma (3—6). The mean absolute amounts of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole in the dialysis fluid (6 liters) were 727 pmol and 206 pmol, respectively (4). The concentrations of the carcinogenic heterocyclic amines in human plasma seem to be well below those causing significant effects on the platelet response to 5-hydroxytryptamine or on monoamine oxidase activities. However, as mentioned in our previous report (7), many factors, such as the in vivo tissue distribution and accumulation of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, remain to be investigated before the potential of tryptophan pyrolysis products for affecting cells such as platelets in vivo can be determined.

In conclusion, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, carcinogenic tryptophan pyrolysates, are potent inhibitors of the platelet aggregation response to 5-hydroxytryptamine, 5-hydroxytryptamine uptake by platelets and monoamine oxidase-A activity.

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